REMARKS

While the status of claims 8-11 is withdrawn, it is respectfully submitted that the Examiner will recognize that there is now a allowable generic or linking claim present and it is respectfully requested that these claims be examined and allowed.

The courteous telephone interview granted by Examiner Snay to the undersigned is acknowledged with appreciation.

During the interview, it was pointed out that "x" and "n" refer to the "number" of values rather than an undefined variable. It was agreed that amending the claims to so state would eliminate the rejection of claims 7 and 20 under 35 USC 112. This has been done above.

It was also pointed out that claim 19 called for continuous manipulation whereas claim 1 did not do so. The Examiner questioned whether it was possible to continuously quantitatively determine the sample if the data is not being continuously manipulated. The Examiner's point is well taken and therefore claim 19 has been cancelled. Also in view of the Examiner's point, claim 1 has been amended to so state.

An obvious typographically error in claim 20 (last subscript in the claim should be "e") has been corrected.

Claims 7 and 20 were asserted to be duplicates. It is respectfully submitted that they are not. Claim 7 recites calibrating whereas claim 20 assumes calibration was previously done.

The claims were rejected under 35 U.S.C. § 103 based on Schafer in view of Tosa and Carter or over Schafer in view of Tosa and Sutherland. This rejection is respectfully traversed.

During the interview, applicants noted that one of the basic differences between the invention and Schafer, not cured by the secondary references, was that Schafer provides only a single value for the unknown's concentration while the invention provides many values. The following points were made previously or during that interview.

The present invention relates to assays which are conducted such that a component, for instance a labeled ligand, becomes at least partially bound to a solid body which can be, for instance, an optical waveguide. As a result of the binding, there is a change in an analyte dependent parameter, for example, a fluorescence emission which is associated with the labeled ligand, which can then be measured.

In the prior art methods, such assays are calibrated using known concentrations of ligands and standard curves. An unknown concentration of ligand can subsequently be determined using the standard curves and in such prior art methods, the unknown concentration of the ligand is determined only once.

The Applicants recognized that during the course of these assays, a reliable measurement of the bound or absorbed component, i.e., without interference from the free components in the assay system, can be obtained by direct continuous monitoring of the component. Thus, an important aspect of the current invention is that, in contrast

to the prior art, an unknown sample is determined continuously. The advantages of such a method are described, *inter alia*, on page 4, lines 6-17. One of the advantages of the invention is that there is an ability to obtain an indication of the unknown concentration of ligand at an early stage in the assay and the ability to spot and avoid random errors.

Schafer is concerned with overcoming the problems which are inherent in "Heidelberger" non-monotonous calibration curves. See col. 1, line 62 et seq. There is no direct correlation in such curves between the concentration of analyte and the measured parameter, e.g., absorbance. Figures 1 and 2 of Schafer illustrate the problem. In Figure 1, it can be seen that there is no direct correlation between concentration and absorbance of a particular sample after 300 seconds (note the curves for concentration C1 and C3 cross). When the same data is plotted in the form of concentration C against a measure of the absorbance X (Figure 2), a hook-shaped "Heidelberger" curve is obtained. As apparent, an unknown sample which has an absorbance X might have one of two different concentrations, one of which falls in the part of the curve labeled "A" and the other falls in the part of the curve labeled "B". For instance, it can be seen that an unknown sample with an absorbance X of 0.8 might have a concentration of either 0.5 or 4.

Schafer provides a solution to this problem by providing a multi-step process. First, a number of "training runs" are carried out in which a property associated with the sample, S(t), is plotted against time for a number of different concentrations. S can be, for instance, absorbance of the sample or a valued derived from the absorbance of

the sample, for example, the difference between absorbance after 12 seconds and 300 seconds (see example 1, col. 11, lines 18-20). This is, in essence, a calibration step shown by Figure 1. It can be loosely said that the data is apparently measured in a "continuous" manner but, as apparent from col. 7, lines 23-26, the data is not measured "continuously" but rather is measured at discrete time points.

In the next step, at least one set of data ("discriminators") is derived from a graph. The data or discriminators are merely variables based on physical properties of the sample such as its absorbance, slope of the graph at a particular point, curvature of the graph, and the like. In example 1 (col. 11, line 39), the discriminator chosen are five gradients of the graph, taken at the time points t1/t2, t2/t3, t3/t4, t4/t5, and t5/t6 and the fifth absorbance value. These data points are contiguous and they might therefore be said to represent continuous data that has been derived or manipulated from the analyte dependent parameter.

A statistical algorithm is derived which uses the discriminators as variables. "Scores" are given to each of the concentrations of the standard samples on the basis of whether or not the concentrations fall in part A or part B of the "Heidelberger" curve (Figure 2). A "boundary score" is determined at dividing the scores whose concentration fall into part A of the curve from those which fall into part B of the curve.

The foregoing steps are repeated in order to refine the algorithm (the "operative discrimination algorithm") which describes the relationship between concentrations of the standard samples and the discriminators. In example 1, the

algorithm makes use of 5 contiguous gradients. This completes the calibration of the assay.

In an analysis, an unknown sample is tested. First, a graph of absorbance or some other physical property of the sample against time is plotted for the sample of unknown concentration in the same manner as the calibration process. Using the "operative discrimination algorithm" from the calibration, an "analysis score" is given to the unknown sample. However, it will be appreciated that the "analysis score" itself is a single value which is derived from the graph of the unknown sample even though the "operative discrimination algorithm" that is used to obtain the "analysis score" might use data from contiguous time points.

Next, the "analysis score" is compared to the "boundary score" to determine whether the "analysis score" falls into part A or part B of the curve. The concentration of the unknown samples is thus determined from knowledge of its absorbance and the calibration curves and information from the determination.

It will be appreciated from the foregoing that the concentration of the unknown sample is obtained in a relatively standard manner, merely by reading off a single concentration value that corresponds the physical property X from the standard curves. The only "continuous" data which arguably derived from the initial data is that which is used in the "operative discrimination algorithm", which used to determine whether the concentration value obtained from the standard graph falls within part A or part B of the curve. Thus, any derived "continuous" data does not itself "determine an unknown sample" but rather is used only to assist in distinguishing between one of

two possible values which the single determination of the unknown sample can take. In the claimed invention, the unknown sample is determined continuously.

Schafer's method is primarily concerned with distinguishing the results obtained from assays that produce "Heidelberger curves". It is not concerned with obtaining results from assays which have not yet reached equilibrium. Note that at col. 6, lines 30-33, Schafer notes that it is common practice to use as the input variable an "end point or plateau value". Another reference to this plateau value is found in the last paragraph in column 5. This teaches that Schafer's method relates to the measurement of an end point value, an equilibrium value from which the concentration of the unknown sample is determined. In the claimed invention, there is continuous determination before equilibrium is established.

During the interview, the Examiner referenced a passage from Schafer which starts at column 9, line 63, and relates to the "analysis run" i.e., the determination of the concentration of the unknown sample, essentially stating that a property of the unknown sample ((S)t) is tested against time in the same way as the calibration samples were tested. Applicants pointed out that the "operative discrimination algorithm" is applied to the data provided in the graph and then "**an** analysis score is calculated from [the data from the graph] . . . ." (emphasis added) and that this passage does not disclose the continuous quantitative determination of an unknown sample. The Examiner requested that Applicants amplify why the word "an" does not refer to each of a plurality the measuring times. In that connection, the Examiner is respectfully requested to consider the following.

Schafer's basic method is described in column 4. Essentially, a plurality of calibration curves are plotted using samples of known concentration in the "training run". For example, a number of values of the absorbance (S) at different times (t) are plotted for a number of samples of known concentration. Figure 1 shows the type of calibration curves that are produced.

From these calibration curves, a standard curve of absorbance v. concentration can be produced, such as that given in Figure 2 of Schafer. This standard curve permits the concentration (C) of an unknown sample with an absorbance (X) to be determined. The problem that Schafer addresses is that the standard curve is bell-shaped and therefore the same absorbance (X) value can represent two different concentration values. Schafer addresses this problem using an "operative discrimination algorithm".

During Schafer's "analysis run", a curve is plotted using the unknown sample (see column 4, line 33). In order to produce this curve, a number of values of the absorbance (S) at different times (t) are plotted for the sample of unknown concentration. Thus, column 9, line 65 et seq, states:

> The kinetic S(t) is determined on a sample to be analysed. If the measurement takes place at discrete measuring times t_i, the measuring times ... should coincide in the analysis run with the measuring times used in the training run".

This passage is stating that, in the analysis run, a curve of absorbance (S) against time (t) is produced in a similar manner to that of the training run, but using the unknown. It refers to the possibility of the absorbance being read at more than one measuring time.

Thus, a curve similar to those illustrated in Figure 1 might be produced in the analysis run, and might well be based on absorbance data taken at a number of different times. It is important to recognize that this information is merely used to produce the curve; it does not directly give the concentration of the unknown.

The quoted passage also refers to the possibility of the measuring times in the analysis run coinciding with those of the training run. This is to ensure that the values obtained in the analysis run correlate to the best extent possible with those of the training run (making the results of the operative discrimination algorithm more accurate).

From the steady state absorbance (X) of the unknown, two possible concentrations (C) can be read off the bell-shaped standard curve (e.g. Figure 2). The purpose of the operative discrimination algorithm is to determine which of these two concentrations is correct. It is a mathematic function which is derived from the calibration curves using a standard statistical technique. The algorithm describes the relationship between the concentration (C) of the known samples at various times (t). (See column 7, line 67, to column 8, line 39). The algorithm provides a single value (a "score") from which it is possible to assign a given absorbance (X) to part A of the bell curve shown in Figure 2 or to part B. In this way, the concentration of the unknown can be determined unambiguously. The mathematics of the statistical technique are not described in detail in Schafer; and in any event, they are irrelevant to the current discussion. (Some of the equations given in Schafer are even wrong: for example

formula 1.2 at column 7, line 40, should read "α_i = arctan (...)", but as said, this is totally irrelevant to the issue under consideration).

The specification of Schafer makes it abundantly clear that only one concentration for the unknown sample is ultimately produced. In this regard, reference may be made to the following: **check**

> "and thereby to achieve an unambiguous correlation to a particular concentration C:". Column 4, lines 1-3
>
> "c.d the input variable X and from the latter the concentration C is determined ..."; Column 4, lines 39-40
>
> "determining the concentration C from the input variable X, ...". Claim 1, step (3)(e)

The above passages, *inter alia,* make it entirely clear that Schafer's method is ultimately only concerned with a determination of a single correct value for the concentration for the unknown. It is solely devoted to determining which of the two possible concentrations (which are derivable from the standard curve, e.g. Figure 2) are correct.

In summary, the basis steps in the Schafer method are as follows:

1. Prepare calibration curves using various known concentrations of sample (e.g. Figure 1).

2. Produce bell-shaped standard curve of absorbance v. concentration (e.g. Figure 2).

3. Produce algorithm which allows the assigning of a particular absorbance (i.e. that of the unknown) to part A or part B of the standard curve.

4. Produce curve of concentration v. time for the unknown.

5. Using standard curve produced in step (2), assign two possible concentrations to the unknown (i.e. one from Part A of the standard curve and one from Part B).

6. Using the algorithm from step (3), determine which of the two possible concentrations of the unknown is correct.

Clearly, Schafer's method results in the production of a SINGLE correct value for the concentration of the unknown.

This is in direct contrast to the currently-claimed method wherein the concentration of the unknown is "continuously quantitatively determined ... for a period of time after the onset of incubation ...". An example of this continuous quantitative determination is given in Figure 2 of the present application. In this Figure, it can be seen that 24 concentration determinations of the unknown sample were made at 2 minute intervals. In this way, an early value for the concentration can be obtained, before the assay reaches a substantially steady state. This is a significant advantage over the prior art methods.

The secondary references do not cure basic deficiencies in Schafer vis-à-vis the claimed invention. Thus, the Tosa reference is only relied on for the purpose of disclosing an assay involving an immunochemical binding reaction on a waveguide surface in order to enable to monitoring of a reaction by luminescence detection. In addition, there is no motivation for combining Tosa with Schafer for reasons discussed in detail in an earlier response.

The Carter reference was cited as an example of providing a waveguide surface for specific binding assays as providing evidence that the heterogeneous technique was well know for providing a number of advantages. It is not seen where this additional reference adds anything further to the disclosure of Tosa but, in fact, it is submitted that this shows there was a lack of motivation. If the use of heterogeneous assay such as those described in Carter (1986) were an obvious substitution, then Schafer (filed in 1993) would have either made the substitution or suggested the assays described were also applicable to heterogeneous assays.

Southerland also does not cure any of the deficiencies in the basic combination of references. It relates to the use of an optical waveguide for optically ascertained parameters of the species in liquid analyte. It, like Tosa, always uses steady state measurements to establish a relationship between those values.

In light of all of the foregoing considerations, it is respectfully submitted that the Examiner will recognize on further consideration that the claims of this application satisfy the requirements of 35 U.S.C. § 103.

The allowance of this application is respectfully solicited.

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Respectfully submitted,

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